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ORIGINAL ARTICLE

Impact of glucocorticoid receptor gene (NR3C1) polymorphisms in Turkish patients with metabolic syndrome

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Abstract
Background The metabolic syndrome (MetS) is characterized by a cluster of metabolic factors, including insulin resistance and type-2 diabetes, abdominal obesity, dyslipidemia, hypertension and microalbuminuria. Impaired glucocorticoid receptor (GR) activity also plays an important role in the etiology of MetS. The objective of our study is to evaluate the effects of GR gene polymorphisms (*Bcl*I, N363S, *Tth*III1 and ER22/23EK) in Turkish patients with MetS.
Materials and methods Seventy subjects with MetS and 185 healthy controls were enrolled in the study. PCR–RFLP analysis was used for genotyping. Results for each polymorphism have been verified by allele-specific oligonucleotide analysis.
Results BclI GG genotype was significantly associated with an increased risk of MetS ($p = 0.02$). Also, only in women, the G allele carriers were significantly associated with higher C-peptide. T allele carriers of *Tth*III1 polymorphism were significantly associated with higher C-peptide, triglyceride, insulin and C-reactive protein (CRP, p value 0.048, 0.022, 0.005 and 0.022, respectively), and lower fasting blood glucose (FBG, $p = 0.02$). The combined carriers of BclI polymorphism G allele and TthIII1 polymorphism T allele were significantly associated with higher diastolic blood pressure in all patients, and lower FBG and postprandial blood glucose in only men. All the ER22/23EK polymorphisms coexisted with polymorphic variant of TthIII1 ($p = 0.0058$).
Conclusion The presence of homozygote polymorphic variant of *Bcl*I might be good predictive markers for the disease susceptibility. The BclI and the TthIII1 polymorphism are associated with sex-specific clinical parameters. Our findings also suggest that the combination of BclI and TthIII1 polymorphisms may play a protective role in blood glucose.

Keywords Metabolic syndrome · Glucocorticoid receptor gene · Glucocorticoid

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Introduction

The metabolic syndrome (MetS) is characterized by a cluster of metabolic factors, including insulin resistance and type 2 diabetes, abdominal obesity, atherogenic dyslipidemia, raised blood pressure, proinflammatory state and prothrombotic state [1, 2]. Glucocorticoid hormones may play an important role in the regulation of metabolic syndrome components. Effects of GC hormones are generally

mediated by the glucocorticoid receptor (GR) [3]. Genetic variations in GR gene (NR3C1) have a potential effect on the GR receptor protein characteristic and expression. This suggests that GR gene polymorphisms are associated with differences in GC sensitivity and in metabolic parameters [4–6]. In early studies, polymorphism of GR gene was the BclI polymorphism (rs41423247). BclI restriction fragment length polymorphism (RFLP) was identified as a C→G nucleotide change in 646 nucleotide downstream of exon 2 (intron 2) [5, 7, 8]. G allele of this Bc*l*I polymorphism was associated with increased sensitivity to GCs [5], increased abdominal obesity [9] and hyperinsulinemia [8]. Another polymorphism (N363S, rs6195) was identified in codon 363 of exon 2 of the GR gene. This AAT to AGT nucleotide change in the codon 363 results in an asparagine to serine amino acid change and this amino acid replacement may change the interactions of the receptor with transcription cofactors [10, 11]. This AGT codon was found to be associated with a higher sensitivity to GCs in vivo [10], obesity and overweight [12], but no association with hypertension and type 2 diabetes has been shown yet [12]. However, N363S polymorphism has not been found in Japanese and Chinese populations [13–15]. ER22/23EK polymorphism (rs6189 + rs6190), which consists of single nucleotide substitutions in codon 22 and 23 [GAGAGG (GluArg, or ER) → GAAAAG (Glu-Lys, or EK)], is known to be associated with relative glucocorticoid resistance [4]. This polymorphism was associated with better survival, as well as lower C-reactive protein levels [16]. Another polymorphism, described as a TthIII1 restriction fragment length polymorphism (rs10052957) in 5′ untranslated region of GR gene, was found to be associated with changes in basal cortisol secretion in men [17]. However, TthIII1 polymorphism might be functionally relevant only in combination with ER22/23EK [18].

The objective of our study is to evaluate the effects of glucocorticoid receptor (GR) gene polymorphisms (BclI, N363S, TthIII1 ve ER22/23EK) in Turkish patients with MetS.

Materials and methods

Study samples

Seventy subjects with MetS using the National Cholesterol Education Program (ATPIII) diagnosis criteria [2] and 185 healthy controls were enrolled in the study. Three or more of the following criteria had to be met: abdominal obesity (men >102 cm, women >88 cm), high triglyceride levels (≥150 ml/dl), low HDL level (men <40 mg/dl, women <50 mg/dl), elevated blood pressure (systolic >130 mmHg, diastolic >85 mmHg), and high fasting glucose (>110 mg/dl). BMI, body fat percent, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, fasting and postprandial glucose as well as hormone levels (cortisol, suppressed cortisol, dehydroepiandrosterone (DHEA), insulin, C-reactive protein (CRP), C-peptide) were determined. The study was approved by Marmara University School of Medicine Ethics Committee (MAR-YÇ-2007-0261).

Measurement of risk factors

Body weight, height and waist-to-hip ratio of the subjects were measured. Body mass index (BMI) and body fat content were calculated by a TANITA body fat analyzer (Tanita Corporation, Tokyo, Japan). Blood samples were collected after 12 h fasting. Serum lipid levels were analyzed using standard procedures. LDL-C was calculated using the Friedewald formula, LDL-C = total cholesterol − (HDL-C + 0.2 × triglyceride). Serum insulin was determined by ELISA using commercial kits (Monobind Inc, Lake Forest, CA, USA) with an automated EIA analyzer (Bio-Rad Laboratories, Hercules, CA, USA). The standard low dose overnight dexamethasone suppression test was employed as described previously. Briefly, 1 mg of dexamethasone was administered at 23:00 h. Blood samples were taken at 08:00 h the next morning and then serum cortisol levels were measured by radioimmunoassay (Amersham Pharmacia Biotech, TFB Co., Tokyo, Japan).

Genotyping

Genomic DNA was isolated from peripheral blood samples of all subjects using standard techniques (phenol–chloroform technique) [19]. DNA samples were investigated for GR gene polymorphisms using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) analysis.

PCR amplification of the GR gene regions was carried out using primer sequences as previously described [20, 21]. For BclI, the forward primer was 5′-TGC TGC CTT ATT TGT AAA TTC GT-3′, and the reverse primer was 5′-AAG CTT AAC AAT TTT GGC CAT C-3′. PCR products (335 bp) were digested at 55 °C for 3 h with 10 U of BclI restriction enzyme (Fermentas, Lithuania). For N363S, the forward primer was 5′-AGT ACC TCT GGA GGA CAG AT-3′, and the reverse primer was 5′-GTC CAT TCT TAA GAA ACA GG-3′. PCR products were digested at 65 °C for 3 h with 10 U of TasI (TspEI) restriction enzyme (Fermentas, Lithuania). For ER22/23EK, the forward primer was 5′-GAT TCG GAG TTA ACT AAA AG-3′, and the reverse primer was 5′-ATC CCA GGT CAT TTC CCA TC-3′. PCR products were digested at 37 °C for 6 h with 10 U of MnlI restriction enzyme (Fermentas, Lithuania). For TthIII1, the forward primer was 5′-TCC AGG

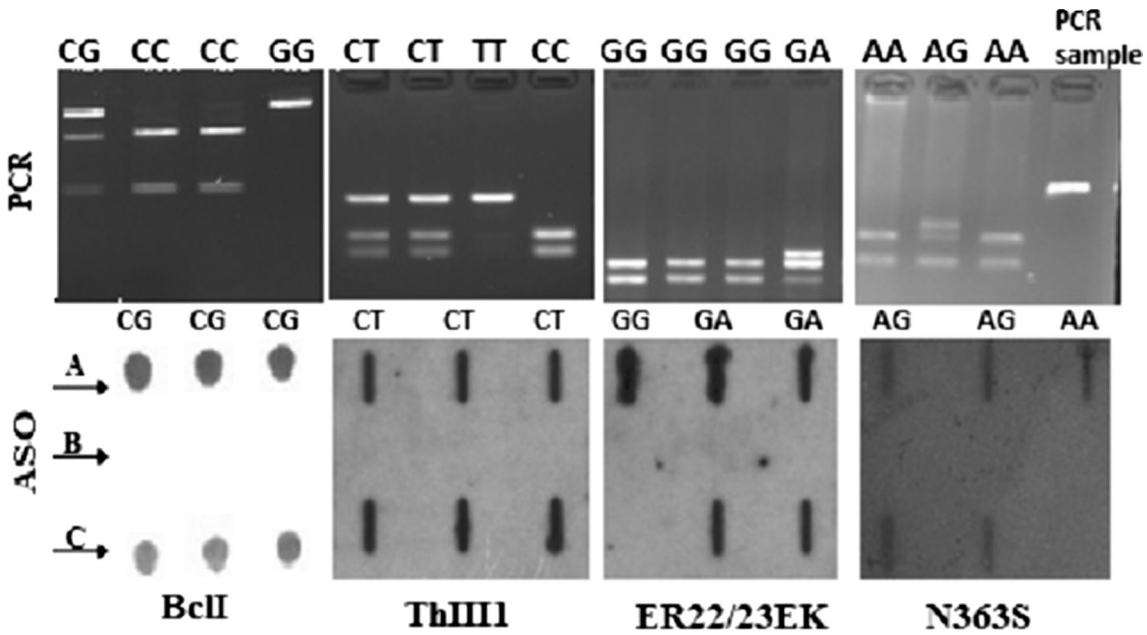

Fig. 1 Genotyping of GR gene polymorphisms. *Top row* RFLP, *bottom row* schematic of dot blot for polymorphisms using wild-type allele ASO prob. Bcl I-*top row*: *lane 1* indicates the CG genotype (335, 221 and 117 bp), *lanes 2* and *3* indicate the CC genotype (221 and 117 bp), *lane 4* indicates the GG genotype (335 bp). TthIII1 *top row*: *lanes 1* and *2* indicate the CT genotype (96, 53 and 43 bp), *lane 3* indicates the TT genotype (96 bp), *lane 4* indicates the CC genotype (53 and 43 bp). ER22/23EK *top row*: *lane 1* indicates the GG genotype (162 and 143 bp), *lane 4* indicates the GA genotype (178, 162 and 143 bp). N363S *top row*: *lanes 1* and *3* indicate the AA genotype (135 and 95 bp), *lane 2* indicated the AG genotype (154, 135 and 95 bp), *lane 4* indicated N363S-PCR sample (248 bp). *Bottom row*: *a* wild type, *b* homozygote mutant and *c* heterozygote. ER22/23EK-*bottom row lane 1* and N363S-*bottom row lane 3* homozygote wild type, *other all lanes* heterozygote

AGT GGG ACA TAA AGC T-3′, and the reverse primer was 5′-CTT AGA AGC AGA GGT GGA AAT GAA G-3′. PCR products were digested at 37 °C for 16 h (overnight) with 10 U of TthIII1 (PsyI) restriction enzyme (Fermentas, Lithuania; Fig. 1).

Results for each polymorphism have been verified by allele-specific oligonucleotide (ASO) analysis. In this method, first 3′-end of wild-type and mutant-type probes was marked with digoxigenin using DIG Oligonucleotide 3′-End Labeling Kit, 2nd Generation (Roche, Germany). Labeling was performed in a 20 µl reaction mixture containing 100 pmol oligonucleotide, 1× reaction buffer, 5 mM $CoCl_2$, 0.05 mM DIG-ddUTP and 20 U/µl terminal transferase, and incubated at 37 °C for 15 min. After incubation, 2 µl 0.2 M EDTA (pH 8.0) was added to stop the reaction. Five microliters of PCR products were mixed with 195 µl reaction mixture containing 10 µl 200 mM EDTA, 80 µl NaOH and 105 µl distilled water (dH_2O), and denaturated at 99 °C for 10 min. After denaturation, the mixed solution was immediately cooled on ice. After rinsing in dH_2O for 10 min the membrane, aliquots (200 µl) were then applied onto a membrane to create 24-slot blots in a hybrid slot-blot apparatus (Biometra, Germany). The membrane was dried overnight at 70–75 °C in oven. Later, DIG Easy Hyb Granules, DIG Wash and Block Buffer Set Kits were used for Hybridization. After the membrane was immersed in 0.2× SSC for 2 min, blots were prehybridized at 54 °C for 45 min (hybridization oven, Model HB-1 Hybrideser-Techne, UK) in hybridization buffer (5× SSC with 0.2 % SDS). Hybridization with the ASO probe was carried out for 4 h. The membrane was the washed two times with 0.2× SSC containing 0.2 % SDS at 42 °C for 10 min. Finally, visualization was done by DIG Luminescent Detection Kit (Fig. 1).

Statistical analysis

Statistical analysis was performed using SPSS 16.0 and GraphPad-Prism5. Chi square analyses, followed by Fisher's exact test wherever required, were used to compare the frequencies of GR gene polymorphisms between patients and healthy controls. The test was also applied for identifying the deviations from the Hardy–Weinberg proportion. Independent samples *t* test, unpaired *t* test, Mann–Whitney *U* test and Kruskal–Wallis test were used to compare clinical parameters and GR gene polymorphisms. Odds ratios with 95 % confidence intervals were given wherever appropriate. All tests were two-tailed, and results were considered significant when p value was <0.05.

Table 1 Comparison of clinical parameters between individuals with and without MetS

Variable	MetS ($n = 70$) (mean ± SD)	Controls (mean ± SD) (n)	p
Age (years)	35.3 ± 10.4	34.54 ± 7.5 (109)	0.588
BMI (kg/m^2)	39.91 ± 5.9	24.4 ± 5.2 (107)	<0.001
TC (mg/dl)	220 ± 35.3	191.1 ± 36.8 (99)	<0.001
TG (mg/dl)	291 ± 150.3	106.4 ± 55.7 (99)	<0.001
HDL-C (mg/dl)	39.9 ± 10.1	47.9 ± 11.4 (99)	<0.001
LDL-C (mg/dl)	129.2 ± 28.3	120.9 ± 36.4 (99)	0.138
Insulin (μU/ml)	30 ± 18.6	9.5 ± 8.8 (94)	<0.001
SBP (mmHg)	152.7 ± 17.3	109.8 ± 15 (22)	<0.001
DBP (mmHg)	96.6 ± 8.8	72.7 ± 9.8 (22)	<0.001
CRP (mg/dl)	6.3 ± 2.1	3.4 ± 1.6 (22)	<0.001
C-peptide	6.3 ± 2.1	2.5 ± 0.8 (22)	<0.001

Significant level = $p < 0.05$ by independent samples t test (two-tailed)

BMI body mass index, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TC* total cholesterol, *TG* triglycerides, *SBP* systolic blood pressure, *DBP* diastolic blood pressure

Results

Comparison of clinical parameters between individuals with and without MetS

Medians for age, anthropometric parameters and biochemical markers in MetS and control subjects are shown in Table 1. The age difference between the MetS and control groups was not significant. HDL-C levels of MetS group were significantly lower than the control group ($p < 0.001$). As expected, there was a statistically significant difference in BMI, systolic and diastolic BP, total cholesterol, triglycerides, insulin, CRP and C-peptide between cases and controls ($p < 0.001$).

Allele and genotype frequencies of GR polymorphisms in MetS and control

Blood samples from 70 subjects with MetS and 185 healthy people were analyzed to determine the frequency of GR gene polymorphisms. The allele frequencies in the studied patients and controls are listed in Table 2. Mutant allele frequencies in the control group were 25.1, 35.1, 2.4 and 0.5 % for the BclI, TthIII1, ER22/23EK and N363S polymorphisms, respectively. This distribution in the patient group was 32.9, 31.4, 1.4 and 0.8 %, respectively.

The genotype distributions in the studied patients and controls are listed in Table 2. BclI polymorphism genotype in the patients was distributed as 32 (45.7 %) with wild type, 30 (42.9 %) with heterozygote and 8 (11.4 %) with homozygote mutant, while as 97 (53.3 %), 77 (42.8 %) and 7 (3.9 %) in the controls, respectively. Furthermore, homozygote carriers of this polymorphism were significantly higher in MetS patients than controls ($p = 0.0234$; Table 2). These results indicate that when compared with combined CC/CG genotype, GG genotype of BclI polymorphism was significantly associated with an increased risk of MetS.

TthIII1 polymorphism genotype in the patients was distributed as 33 (47.1 %) with wild type, 30 (42.9 %) with heterozygote and 7 (10.0 %) with homozygote mutant, while they were as 75 (40.5 %), 90 (48.6 %) and 20 (10.8 %) in the controls, respectively. ER22/23EK-carriers was determined as 1 (1.4 %) in the patient group and 9 (5.0 %) in the controls. N363S polymorphism genotype in the patients was distributed as 65 (98.5 %) with wild type and 1 (1.5 %) with heterozygote, while as 183 (98.9 %) and 2 (1.1 %) in controls, respectively. Moreover, all the ER22/23EK polymorphisms coexisted with polymorphic variant of TthIII1 ($p = 0.0058$; Table 3). The carriers of the N363S variant allele were identified in 1.5 % of the patients and in 1.1 % of the control group (Table 2). Additionally, while G allele for N363S and ER22/23EK polymorphism was rare, homozygote carriers of N363S polymorphism were not found in our population.

Individual effects of GR polymorphisms in clinical parameters of MetS patients

Both genetic polymorphisms N363S and ER22/23EK of GR gene were not individually associated with clinical parameters of MetS patients. On the other hand, BMI was significantly higher in heterozygous carriers (4.9 %) compared with wild-type subjects of ER22/23EK polymorphism in the control group ($p = 0.011$). No significant differences in clinical parameters between BclI genotype groups were present. However, the G carriers of BclI polymorphism showed a significant higher C-peptide level in comparison with CC homozygote group in women ($p = 0.01$; Fig. 2a), but not associated with any clinical parameter in men.

T allele carriers of TthIII1 polymorphism were significantly associated with C-peptide, triglyceride, insulin, FBG and CRP levels in all patients with MetS (Table 4). Moreover, T allele carriers of this polymorphism had a higher level of insulin, CRP, C-peptide and lower level of LDL-C, PPBG than non-carrier group in men (Table 4), but not associated with any clinical parameters in women. Additionally, insulin levels were significantly different between three genotypes (CC, CT and TT) and it was highest in TT genotype ($p = 0.0183$; Fig. 2b).

Table 2 Genotype and allele frequencies of GR polymorphisms in MetS and control subjects

	Genotype frequencies				Allele frequencies			
	Genotype	Patients, *n* (%)	Controls, *n* (%)	*p* value	Allele	Patients, *n* (%)	Controls, *n* (%)	*p* value
Bcl I	CC	32 (45.7)	97 (53.3)	0.0704*	G allele	94 (67.1)	271 (74.9)	0.082
	CG	30 (42.9)	77 (42.8)		G allele	46 (32.9)	91 (25.1)	
	GG	8 (11.4)	7 (3.9)					
Not detected	C carriers	62 (88.6)	174 (96.1)	**0.0234**		–	8	
Total	Non-C	8 (11.4)	7 (3.9)			140 (100)	370 (100)	
		–	4					
		70 (100)	185 (100)					
Tth III	CC	33 (47.1)	75 (40.5)	0.4197*	C allele	96 (68.6)	240 (64.9)	0.4307
	CT	30 (42.9)	90 (48.6)		T allele	44 (31.4)	130 (35.1)	
	TT	7 (10.0)	20 (10.8)					
Not detected	C carriers	63 (90)	165 (88.9)	0.851		–	–	
Total	Non-C	7 (10)	20 (11.1)			140 (100)	370 (100)	
		–	–					
		70 (100)	185 (100)					
ER22/23EK	GG	69 (98.6)	176 (95.1)	0.5186*	G allele	138 (98.6)	361 (97.6)	0.7352
	GA	0 (0.0)	9 (4.9)		A allele	2 (1.4)	9 (2.4)	
	AA	1 (1.4)	0 (0.0)					
Not detected	G carriers	69 (98.6)	185 (100)	0.2745		–	–	
Total	Non-G	1 (1.4)	0 (0)			140 (100)	370 (100)	
		–	–					
		70 (100)	185 (100)					
N363S	AA	65 (98.5)	183 (98.9)	0.7806*	A allele	131 (99.2)	368 (99.5)	1
	AG	1 (1.5)	2 (1.1)		G allele	1 (0.8)	2 (0.5)	
	GG	0 (0)	0 (0)					
Not detected	A-carriers	66 (100)	185 (100)	–		8	–	
Total	Non-A	0 (0)	0 (0)			140 (100)	370 (100)	
		4	–					
		70 (100)	185 (100)					

Significant level = $P < 0.05$ (bold) by Fisher Exact test (column value <5), Chi square test (column value >5)

* Chi square test for trend (three rows)

Table 3 Association between ER22/23EK and TthIII1 polymorphism ($p = 0.0058$)

TthIII1	ER22/23EK		Total
	Wild type	Polymorphic allel	
Wild type	108 (100 %)	0	108
Polymorphic allel	137 (93.3 %)	10 (6.7 %)	147
Total	245 (96 %)	10 (4 %)	255

Significant level = $p < 0.05$ by Fisher Exact test

Genotype combinations of GR gene polymorphisms in MetS patients and controls

The genotype combinations of four GR gene polymorphisms were analyzed in each individual (Table 5). The five most prevalent genotypic combinations were presented separately and the remaining rare combinations were pooled together. The CG+GG–CC–GG–AA genotype (BclI, TthIII1, ER22/23EK and N363S, respectively;

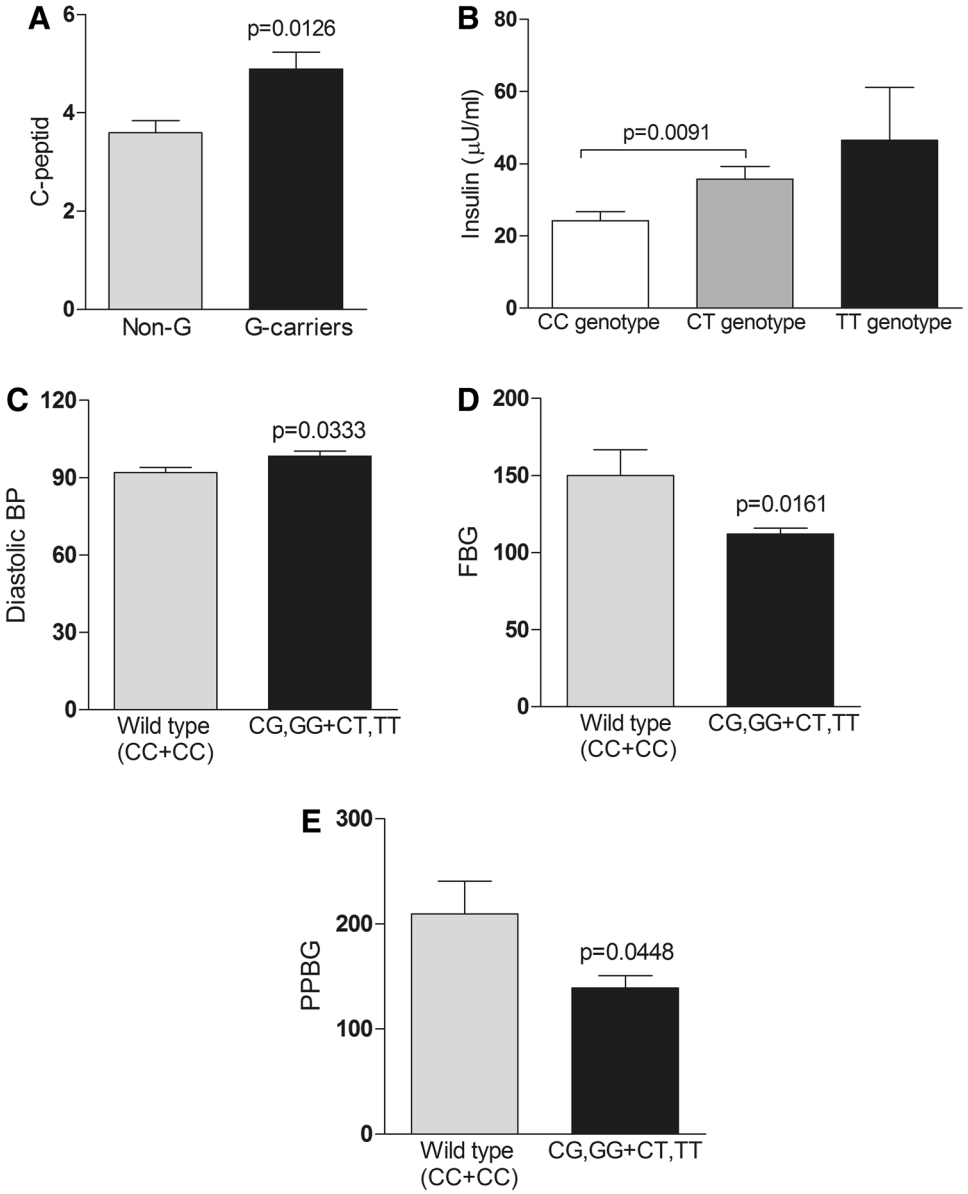

Fig. 2 Comparison of clinic parameters with GR gene polymorphisms. *Diastolic BP* diastolic blood pressure, *FBG* fasting blood glucose, *PPBG* postprandial blood glucose

Table 4 Comparison of clinical parameters with TthIII1 genotypes according to T-carriers vs. non-T carriers

Variable	Men			Men + women		
	T-carrier (CT/TT) (mean ± SEM) (*n*)	Non-T (CC) (mean ± SEM) (*n*)	*p*	T-carrier (CT/TT) (mean ± SEM) (*n*)	Non-T (CC) (mean ± SEM) (*n*)	*p*
Age (years)	30.00 ± 1.565 (25)	32.90 ± 2.355 (20)	0.2952	33.3 ± 9.9 (37)	37.3 ± 10.6 (33)	0.104
BMI (kg/m^2)	41.07 ± 1.103 (25)	39.03 ± 0.7805 (20)	0.1562	40.7 ± .5 (36)	39.6 ± 4.9 (33)	0.425
TC (mg/dl)	203.4 ± 7.370 (22)	217.6 ± 7.228 (20)	0.1784	215.8 ± 35.9 (33)	220.1 ± 30.7 (32)	0.625
TG (mg/dl)	308.7 ± 34.85 (21)	242.7 ± 14.54 (20)	0.0939	320.4 ± 173 (32)	243.2 ± 69.2 (32)	0.022*
Cortisol	17.73 ± 1.298 (21)	15.20 ± 1.237 (20)	0.1664	14.21 ± 1.057 (32)	17.01 ± 1.052 (32)	0.065
DHEAS	317.7 ± 15. 29 (21)	284.9 ± 20.22 (19)	0.2049	307.8 ± 12.89 (32)	284.2 ± 13.50 (31)	0.211
HDL-C (mg/dl)	38.85 ± 2.278 (22)	38.96 ± 2.949 (20)	0.9765	39.9 ± 9.5 (33)	40.1 ± 11.3 (32)	0.952
LDL-C (mg/dl)	113.8 ± 6.273 (18)	133.9 ± 6.919 (19)	0.0387*	121.6 ± 29.4 (26)	134 ± 26.3 (30)	0.101
Insulin (U/ml)	39.83 ± 4.337 (22)	24.53 ± 3.411 (20)	0.0093**	37.1 ± 20.2 (33)	24.2 ± 14.4 (32)	0.005**
SBP (mmHg)	150.1 ± 3.534 (22)	151.6 ± 3.588 (19)	0.777	153.2 ± 19.7 (34)	153.1 ± 14.5 (31)	0.979
DBP (mmHg)	99.32 ± 1.781 (22)	94.74 ± 1.552 (19)	0.0636	98.5 ± 7.3 (34)	995.2 ± 9.4 (31)	0.11
CRP (mg/dl)	7.333 ± 0.5360 (21)	5.890 ± 0.3722 (18)	0.039*	6.9 ± 2.4 (32)	5.7 ± 1.4 (30)	0.022*
C-peptide	6.005 ± 0.4152 (21)	4.250 ± 0.4385 (19)	0.0061**	5.3 ± 2 (32)	4.4 ± 1.6 (31)	0.048*
FBG	120.8 ± 6.008 (22)	148.5 ± 12.86 (20)	0.0509	123.4 ± 5.2 (32)	149 ± 9.8 (32)	0.022*
PPBG	153.2 ± 14.52 (21)	201.8 ± 18.05 (19)	0.041*	183.7 ± 9.2 (58)	163.8 ± 30.4 (4)	0.574

Significant level = $p < 0.05$ by unpaired *t* test (two-tailed)

BMI body mass index, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TC* total cholesterol, *TG* triglycerides, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FBG* fasting blood glucose, *PPBG* postprandial blood glucose

* $p < 0.05$

** $p < 0.01$

Table 5 The frequency of genotypic combinations of polymorphisms in patients and in healthy controls

BclI	TthIII1	N363S	ER22/23EK	MetS (%)	Controls (%)	OR; 95 % CI	p
CC	GG	AA	GG	4 (6)	16 (9)	0.6; 0.20–1.94	0.604
CC	CC	AA	GG	11 (16)	18 (10)	1.7; 0.75–3.79	0.199
CG+GG	CC	AA	GG	19 (27)	19 (11)	3.2; 1.56–6.46	**0.001***
CC	CT+TT	AA	GG	20 (29)	36 (20)	1.6; 0.85–3.03	0.138
CG+GG	CT+TT	AA	GG	16 (23)	32 (18)	1.38; 0.70–2.71	0.349
Rc				0	60 (32)		
Total				70	181		

Significant level = $p < 0.05$ by Fisher Exact test (column value <5), Chi square test (column value >5)

CG+GG CG and GG carriers of BclI polymorphism, *CT+TT* CT and TT carriers of TthIII1 polymorphism, *Rc* remaining combinations

* $p < 0.005$

mutant allele carriers for BclI and non-mutant allele carriers for other three polymorphisms) combination was found to be significantly higher than controls [OR 3.18, 95 % CI (1.56–6.46), $p = 0.001$].

Combined effects of GR polymorphisms in clinical parameters of MetS patients

To evaluate the combined effect of BclI and TthIII1 polymorphisms on clinical parameters, we divided our study population into two groups: a group of non-carriers of both polymorphisms (CC+CC) and a group of carriers of both polymorphisms (CG/GG+CT/TT). DBP of the CG/GG+CT/TT group was significantly higher than the other groups in all patients with MetS ($p = 0.033$; Fig. 2c). In addition, the CG/GG+CT/TT group had a lower level of FBG and PPBG than the other groups in men ($p = 0.0161$ and $p = 0.0448$, respectively; Fig. 2d, e), but differences in the clinical parameters were not statistically significant in women ($p = 0.7715$ and $p = 0.8857$, respectively). We did not find any other combinational effects of GR polymorphisms associated with clinical parameters in our MetS patients.

Discussion

MetS initiating molecular mechanisms are still not fully understood. GCs may influence process leading to metabolic disorders and the action of it at the cellular level is mediated by the GR [22, 23]. Most of the previous studies have focused on the relationship between GR polymorphisms and MetS in most of the populations except Turkish population. Therefore, the present study focused on four GR gene polymorphisms that were associated with metabolic syndrome in Turkish population. We found no significant differences in the frequencies of the three genetic polymorphisms (N363S, ER22/23EK and TthIII1) between the controls and the patient group. The number of homozygote carriers for BclI polymorphism in patients with MetS was significantly higher compared to the control group ($p = 0.0234$). Additionally, the G allele carrier of this polymorphism showed a significant higher C-peptide in comparison with CC homozygote group in women ($p = 0.01$). We found that the T allele of TthIII1 polymorphism is associated with C-peptide, triglyceride, insulin, CRP and FBG in patients with MetS. Furthermore, all the Er22/23EK polymorphisms coexisted with polymorphic variant of TthIII1 ($p = 0.0058$).

Although the BclI polymorphism of the GR gene has been identified in many populations [24–27], N363S and ER22/23EK polymorphisms were not found in Chinese population, Chinese Han population and Japanese population [14, 15, 25, 26, 28]. Our results suggested that A allele of ER22/23EK polymorphism and G allele of N363S polymorphism is rare in our population and this is similar to the results of a study in a Chinese and Japanese population.

In our study, the GG homozygote group showed a significant association with increased risk of MetS in comparison with the C-allele-carrier subjects (CC and CG) and GG genotype was considered as a risk factor for MetS ($p = 0.02$). This finding was consistent with the results of previous study, in which GG genotype was identified to be more frequent in patients with MetS [15]. In another study, BclI polymorphism has not been found significantly different in genotype and allele frequencies comparing obese and normal weight women and the genotype was associated with higher levels of insulin and blood glucose [8]. Our data for the BclI polymorphism in patients with MetS are slightly similar for G allele frequency compared with the data published previously by Koeijvoets et al. and the same study showed that men with the BclI haplotype were associated with cardiovascular disease [29]. However, in our study, no association between this polymorphism and clinical parameters was determined in men. Several studies have suggested that GR gene polymorphisms could have gender-specific effects on anthropometric and metabolic variables [5, 6, 30]. Melcescu et al. [31] showed that while the BclI polymorphism was associated with higher BMI in Caucasian women, it was not associated with BMI in African Americans. There are conflicting results about this polymorphism. Some studies showed an association with high BMI, abdominal obesity, SBP and cortisol levels. In contrary, some studies report that this polymorphism is associated with low BMI and insulin resistance [7, 9]. Also it was reported that BclI polymorphism is associated with low BMI in the elderly individuals [5]. Additionally, Yan et al. [28] showed that only GG homozygotes had higher BMI and SBP and lower plasma glucose and triglycerides. In this study high C-peptide level among homozygous GG carriers than among C allele carriers was found only in women.

The frequency of TthIII1 in the study population of Rosmond et al. [9] (284 middle-aged men) and Van Rossum et al. [18] was: CC 49.6 %, CT 41.4 % and TT 9.0 %, CC 39.7 %, CT 44.5 % and TT 15.8 % compared to CC 47.1 %, CG 42.9 % and GG 10 % in patients with MetS in our study population. Additionally, there was no association between TthIII1 polymorphism and MetS. However, we found that T allele carriers of this polymorphism had a higher level of insulin, CRP, C-peptide and lower level of LDL-C, PPBG than non-carrier group in men, but we did not find an association between this polymorphism and any other clinical parameters. Van Rossum et al. [18] and Yan et al. [28] have suggested that TthIII1 polymorphism itself is not associated with clinical parameters, but our results show that this polymorphism itself is associated with certain clinic parameters related to MetS. Another study showed that heterozygous carriers of this polymorphism in African–Americans with HIV infection had significantly higher levels of high-density lipoprotein cholesterol and a tendency toward lower glucose and triglyceride levels and lower visceral adipose tissue mass [32]. CRP is used as a sensitive marker of inflammation and elevated level of CRP is associated with MetS [33]. A previous study concerning the relationship between CRP and components of the metabolic syndrome indicated that only obesity was significantly associated with higher CRP levels [34]. Our study demonstrated the associations of TthIII1-T allele with higher insulin, CRP, C-peptide, and with lower levels of LDL-C, PPBG for the first time in MetS. We suggest that TthIII1 polymorphism may play a protective role in high cholesterol and blood glucose levels, but it could be a potential risk factor for insulin, CRP and C-peptide.

Taken together both control and patient subjects, we found that ER22/23EK polymorphism coexisted with TthIII1 polymorphism, but there was no carrier of TthIII1 C allele ($p = 0.0058$). Our data supports that ER22/23EK polymorphic allele have arisen de novo on the highly

frequently occurring TthIII1 T allele as indicated in a previous study [18].

In different studies concerning about TthIII1 and ER22/23EK polymorphisms, while ER22/23EK polymorphism was associated with the better metabolic health profile, TthIII1 polymorphism has not been found to be associated with any metabolic parameters [4, 5]. Van Rossum et al. [35] showed that lean body mass was significantly higher in male heterozygous carriers (8 %) of the ER22/23EK variant. Our study demonstrated that A allele of ER22/23EK polymorphism is rare in our population and BMI is significantly higher in heterozygous carriers (4.9 %) as compared with wild-type subjects of this polymorphism in the control group ($p = 0.011$).

We did not find any interaction between TthIII1 T and BclI G allele frequencies in MetS. However, we showed that carriers of both alleles of these polymorphisms had a lower FBG and PPBG in men with MetS. Therefore, our study suggests that the combination of the polymorphisms may play a protective role in blood glucose.

In the present study, we did not find an association of the ER22/23EK and the N363S polymorphisms with MetS. A possible explanation for the lack of an association between these two polymorphisms and MetS could be their low frequency. Therefore, association with clinical parameters of N363S was ruled out in our study group, as both controls and patients were approximately 99 % homozygous wild type for N363S. The N363S data of our study are similar to those reported in populations of Japanese, Chinese, Caucasian and African American [13, 14, 31].

In conclusion, we describe here that the N363S, ER22/23EK and TthIII1 polymorphisms may not be considered as genetic risk factors for MetS in Turkish population. However, the BclI polymorphism might be good predictive markers for MetS in Turkish individuals. We conclude that the BclI and the TthIII1 polymorphisms are associated with clinical parameters in a sex-specific manner. Our findings also suggest that TthIII1 polymorphism and the combination of BclI and TthIII1 polymorphisms may protect from high blood glucose levels. In clinical practice, the GR polymorphism screening during MetS treatment could help to identify the subgroup of patients who are at risk for developing a higher level of insulin, CRP, C-peptide and lower level of LDL-C, PPBG; these patients would benefit most from personalized treatment. Furthermore, the study provides a lead for future investigation for the effects of GR polymorphisms on MetS in this population.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval All the research has been performed according to the Ethical Standards involving human participants.

Informed consent Informed consent was obtained from all individual participants included in the study.

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